

**An Environmental Assessment for An Experimental Test of Genetic
Rescue in Small, Isolated Westslope Cutthroat Populations in the
Missouri River Drainage, Montana**

**ENVIRONMENTAL ASSESSMENT
DECISION NOTICE**

**Montana Fish, Wildlife & Parks
Region Three, Bozeman
Region Four, Great Falls
June 5, 2017**

Proposed Action

Montana Fish Wildlife and Parks (FWP), in collaboration with The University of Montana and the U.S. Geological Survey, has proposed an experimental project to determine the feasibility of improving the genetic fitness of isolated native fish populations. As detailed in the draft Environmental Assessment (EA), the research effort includes transferring a small number of Westslope Cutthroat Trout (cutthroat) between three pairs of streams to determine if the “addition” of new genetic diversity would improve the relative health of the populations, including fish size and population abundance. Results of the study are anticipated to provide fisheries managers with information on the potential utility of “genetic rescue” for long-term conservation of small, isolated native fish populations, including Westslope Cutthroat Trout.

The draft EA identified six specific study streams for the project, including two streams in the Belt Creek (near Neihart, MT), Upper Missouri River (near Townsend, MT), and Big Hole River (near Wisdom, MT) drainages. After release of the EA, fish health concerns were identified in one of the identified project streams, Staubach Creek. Though not known to be present within the cutthroat trout population in the headwaters of Staubach Creek, the detection of whirling disease in brook trout in the lower reaches of drainage resulted in a determination that cutthroat should not be transferred from this stream. This possible situation was described in the draft EA.

An alternate study stream and cutthroat population has been identified to replace Staubach Creek as a fish transfer donor source for the project. The South Fork of Quartz Creek, near Clancy, Montana, maintains a genetically pure, isolated, and health-tested cutthroat population that is a suitable replacement for Staubach Creek. Following the protocols described and analyzed in the draft EA, in lieu of using Staubach Creek as a donor source, cutthroat would be transferred from the South Fork of Quartz Creek into Hall and Staubach Creeks. The South Fork of Quartz Creek would serve only as a donor of fish, and not receive cutthroat from the other populations. The South Fork of Quartz Creek supports several hundred cutthroat trout, and the single removal of up to 16 cutthroat is not considered a significant short or long-term impact to the population. The overall environmental analysis as presented in the draft EA remains the same for use of this stream as a donor source.

Montana Fish, Wildlife & Parks is required by the Montana Environmental Policy Act (MEPA) to assess significant potential impacts of a proposed action to the human and physical environment. In compliance with MEPA, an EA was completed for the proposed project by FWP and released for public comment on April 20, 2017.

Public comments on the proposed project were taken for 43 days (through June 1, 2017). To distribute information on the project an EA notice was mailed to individuals and groups that have previously voiced interest in similar projects in south-west and north-central Montana, and a legal notice of the EA release was printed in the Montana Standard (Butte, MT), Helena IR (Helena, MT), and Great Falls Tribune (Great Falls, MT) newspapers. A draft EA was also posted on the FWP webpage: <http://fwp.mt.gov/publicnotices/>.

One written comment was received via email during the comment period. The comment included four direct questions that requested clarification on the study design and elements of the environmental analysis. The comments are presented on the attached document, and detailed responses are provided.

Decision

Based on the analysis in the draft EA, the public comments received, and benefits and risks associated with this project, it is our decision to go forward with the Proposed Action as outlined in the draft Environmental Assessment. We find there to be no significant impacts on the human and physical environments associated with this project, including the addition of the South Fork of Quartz Creek cutthroat population as a component of the project. Therefore, we conclude that the Environmental Assessment is the appropriate level of analysis, and that an Environmental Impact Statement is not required.

Sincerely,



Sam B. Sheppard
FWP Region Three Supervisor



Gary Bertellotti
FWP Region Four Supervisor

Comments and responses related to the Draft Environmental Assessment “An Experimental Test of Genetic Rescue in Small, Isolated Westslope Cutthroat Trout Populations in the Missouri River Drainage, Montana

Comment 1. *The following paragraph, taken from the “Recommendations from the Westslope Cutthroat Trout Technical Committee for the Genetic Conservation of the Westslope Cutthroat Trout in the Upper Missouri River Drainage (March 1988), directs caution in maintaining population viability and local level adaptations of populations when transplanting native populations as described in the proposed action. The EA should address this issue and identify what the potential risks as well as potential benefits would be to the impacted populations. Or explain why the adverse impacts suggested in the paragraph below need not be considered.*

“The allelic diversity of westslope cutthroat trout also suggests that historically there has been very little gene flow among populations, except possibly at a very local level (Wright 1932). In this situation, even fairly weak natural selection can effectively establish local adaptations. Thus, there is a good possibility that some populations of westslope cutthroat trout may have some degree of local adaptation (e.g. Fox 1993; Phillipp and Clausen 1995) which could be broken down, compromising population viability, if the native fish interbreed with westslope cutthroat trout introduced from other populations. It is likely that westslope cutthroat trout conservation and restoration efforts at times will call for the stocking of fish either from a hatchery broodstock or from transplants from native populations. In view of the above possibility, the potential for these efforts to adversely impact native populations needs to be considered before introductions are made.”

Response:

Restoring gene flow and crossing inbred residents with translocated individuals can reverse many negative effects of inbreeding – a phenomenon termed ‘genetic rescue’ (Vila et al. 2003, Hogg et al. 2006, Bouzat et al. 2009, Hedrick and Fredrickson 2010, Johnson et al. 2010, Frankham 2015, Whiteley et al. 2015, Robinson et al. *in press*). There is substantial evidence that gene flow causes a boost to fitness (Whiteley et al. 2015). These studies range from experimental mixing of populations to pulses of gene flow into natural populations (Whiteley et al. 2015). As of a review conducted in 2015, there were 18 studies that had examined the fitness consequences small numbers of immigrants into natural populations. Specifically, these studies tested for individual fitness effects and whether there was a population response (increase in abundance or population growth rate). Only one of these 18 studies found negative population-level consequences of a small number of immigrants and this was a study of copepods with the specific goal of mixing extremely divergent populations (21% mitochondrial sequence divergence) to test for outbreeding depression (Hwang et al. 2012).

The primary risk of this experiment (outside of disease concerns) is outbreeding depression (OD). Outbreeding depression would occur if source and recipient populations differ in local adaptations they possess. Upon mixing, hybridized individuals could have reduced fitness because local adaptations have been disrupted. Published guidelines predict OD when populations have fixed chromosomal differences, have been isolated for more than approximately 500 years, or inhabit different environments

(Frankham et al. 2011). It has been suggested that concerns about outbreeding depression in recently fragmented populations are ‘almost certainly excessive’ (Frankham et al. 2011). There is limited empirical evidence for OD, outside of the extreme copepod example mentioned above (Hwang et al. 2012) or when life history or phenological differences are large. Within salmonids, there is substantial evidence that environmental mismatches have led to failure of establishment of Pacific salmon populations into unoccupied (but previously occupied) habitat, likely because the translocated individuals lacked local adaptations to the new environment (Wood 1995). There is also evidence for OD when domesticated (hatchery) and wild individuals cross (Tymchuk et al. 2007). Despite the vast evidence for local adaptation in salmonids (Hendry and Stearns 2004), there is evidence for OD in some cases characterized by substantial genetic divergence (Gharrett et al. 1999) but OD tends to be unpredictable and sporadically occurs when genetic distances are smaller (Houde et al. 2011).

The eastside westslope cutthroat trout populations upon which we will focus are not predicted to lead to OD based on Frankham’s guidelines (Frankham et al. 2011). They do not have chromosomal differences, have likely been isolated for fewer than 500 years, and come from similar environments. We acknowledge that the last consideration (environmental similarity) is quite vague. Environmental differences will occur within a paired population but we argue that selection pressures faced by populations within a pair are likely to be similar and to have led to similar local adaptations. These will be headwater sites with relatively similar stream temperature, flow regimes, habitat availability, and predation regimes. However, given the evidence for local adaptation in headwater salmonids, caution is warranted and experimental tests are necessary before genetic rescue is used more widely.

For eastside headwater westslope cutthroat trout populations, local adaptations could occur through a wide variety of traits (growth rate, size and age at maturity, phenology of movement or reproduction, disease resistance, etc). One possibly important local adaptation may involve size and age at maturity. Earlier maturity at smaller body size could be advantageous in these headwater habitats. Since population pairs (source and recipient) are likely to have faced similar selection pressure on size and age at maturity, we do not expect outbreeding depression to act through this trait. Rather, we envision a more likely positive fitness outcome as follows: heterosis might lead to increased growth rate and body size in YOY. Increased body size at the end of the first summer could lead to higher over-winter survival. Increased YOY survival combined with the possibility of restoring additive genetic variation in age at maturity, and thus possibly fueling a further downward shift in age at maturity, could boost population growth rate. Even without an effect on age at maturity, increased YOY survival is expected to boost population growth rate.

Another possible outcome is that we do not induce heterosis. This is an outcome that might lead to lack of a genetic rescue effect (a fitness boost from gene flow) but importantly, would not cause harm to the recipient populations. We do not fully understand the genetic basis of inbreeding depression. It is more likely due to fixation of deleterious recessive alleles than the general effect of loss of heterozygosity. However, we do not know if deleterious recessive alleles associated with inbreeding depression are due to many genes of small effect each, or few of large effect. Because of the many

possible pathways responsible for inbreeding depression, we hypothesize that inbreeding depression is most often due to many genes of small effect. However, we cannot rule out that any given population might suffer from inbreeding depression due to the fixation of a deleterious allele with a major effect on fitness. If the donor and recipient populations happen to be fixed for the same deleterious allele of major effect, we would not see a fitness boost from heterosis. That is, the deleterious allele would not be masked in the heterozygous form in resident x transplant offspring. Our experimental design will allow us to test for this outcome. However, we consider this outcome unlikely and again, there are no predicted negative fitness effects of this outcome.

Comment 2. *The EA needs to more clearly define if the Proposed Action is implemented, how would the study design be structured to determine if changes in abundance can be identified as resulting from the genetic rescue actions versus habitat conditions or other factors that could affect abundance if the changes in abundance are anticipated to be small.*

Response:

We will monitor and quantify the outcome of our genetic rescue experiment in four ways: (1) measure annual reproductive success of offspring produced from resident (i.e., native to the stream) x resident (R x R), resident x transplant (introduced to the stream) (R x T), and transplant x transplant (T x T) crosses by conducting genetic parentage analyses. (2) Measure (and quantify increase) in the proportion of the gene pool from immigrants. (3) Measure demographic performance (survival), abundance, and density with either genetic tagging or pit tags. (4) Measure the effective number of breeders (N_b) (as in Whiteley et al. 2012; Waples et al 2014). We will conduct six years of post-rescue monitoring. We anticipate the production of F2 offspring in 2020 (when fish born in 2017 are age-3+). Following six years of post-rescue monitoring (2017-2022), we will use generalized linear mixed models (GLMMs) to quantify the relative success of each rescue approach given a variety of physical and population level parameters. We will conduct a series of models with family size, body size, or survival as response variables. Predictor variables will be cross-type (fixed effect), family (random effect), and stream (random effect). We will use appropriate error structures in these models, such as a negative binomial for reproductive success (Araki et al. 2007).

We will genotype 400 SNP loci (using Rapture technology; Ali et al. 2015) in each of approximately 250 fish per each of the four focal streams in which we conduct reciprocal transplants. We will generally conduct electrofishing during the summer to target Age-1+ and older fish. We will fin clip all Age-1+ and older fish captured. The only exception to summer sampling will occur in the fall (late September or October) of 2017. We will conduct an electrofishing survey at that time to target F1 Age-0+ (YOY) from the initial 2017 cohort. The purpose of this survey will be to genetically confirm reproduction by transplant and to obtain as large as possible a sample of the initial cohort.

We plan to genotype 250 individuals from 2017 through 2022 (through the production of F2s), thus we will genotype approximately 1,000 fish per year for six years (total of 6,000 fish for the experimental sites). We will genotype an additional 50 fish per year for a total of 300 fish from the control site. We will also genotype the original 48 transplanted adults.

We will use the genotypic data to conduct mark-recapture-based estimates of apparent survival. We will implant PIT tags into Age-1+ fish for individual identification. We will estimate survival from Age-0+ to Age-1+ for the 2017 cohort only, based on genotypic individual identification. Subsequent yearly sampling occasions will allow estimates of survival at a yearly time scale for each cross type (R x R, R x T, and T x T).

Finally, we will monitor several “control” streams that receive no introductions to determine if changes in environmental conditions during the period of the project are having influences on results being observed. That is, we predict a positive effect from genetic rescue on abundance above and beyond that observed in control sites.

We also note that while an increase in abundance is an ultimate goal of this project, an increase in relative fitness through a boost to vital rates is also highly likely to increase population persistence. Thus, if we observe elevated vital rates (e.g. survival to age, fecundity, somatic growth rates) but do not observe an increase in abundance, the experiment will still have been successful.

Comment 3. *The EA seemed to indicate that pure endemic populations would be utilized in the proposed action and did not identify how the populations proposed for use were chosen. The EA should provide information as to whether any of these populations have been replicated and if utilizing a replicated population would be a safer approach if potential results were not beneficial to the viability of the populations.*

Response:

WCT populations were selected based on several criteria including being non-hybridized, adequate spawning habitat available, disease free, reasonably accessible by road or trail, and ~ 50% reduction in heterozygosity relative to average heterozygosity of other eastside populations. Very few WCT populations meet these specific criteria, and while the possible use of “replicated” populations was considered, it was determined that the prior replication of the population into a new stream could result in genetic changes to newly established populations that mask or complicate the analysis of the predicted genetic rescue response. Most of the populations (i.e., NF Little Belt, Hall, SF of NF of Divide, and Papoose Creek) have been replicated by moving individuals of their gametes to other vacant habitats. These efforts provide some assurance that the genetic diversity of these populations would remain conserved should a negative outcome occur from the Genetic Rescue project. Potential risks of the study have been considered and evaluated by fisheries managers, as well as described in the draft Environmental Assessment, and the potential benefit of information learned from the study towards long-term cutthroat conservation was deemed to out-weigh concerns of potential out-breeding depression or any other potential, direct impacts of the study (e.g., electrofishing injury).

Comment 4. *Are the sample sizes proposed for transferring between the six streams adequate for studying conservation genetics with a scientific methodology? Additionally the EA should clearly state the preferred alternative; on page 13 it described “the transfer of 8 Westslope Cutthroat Trout between three pairs of streams (48 fish total)” and on page 4 it stated “The project would include transferring sixteen WCT between pairs of populations (i.e., 8 fish in each direction; 48 fish in total for the project) in the*

summer of 2017". Clearer detail regarding the specifics of translocations would be helpful.

Response:

Yes, per prior discussion and published research, genetic rescue is predicated with the knowledge that the transfer of only a small number of fish between streams is sufficient to boost vital rates that are suppressed by inbreeding depression without disrupting local adaptations. The transfer of more individuals would increase the likelihood that local adaptations are disrupted. The study design follows those of a similar effort with Eastern Brook Trout (Robinson et al. in press), and genetic and population changes were noted in 100% (4) study streams. We will follow the reproductive success of the transplants over time and examine the rate of integration of their genes into the recipient populations. Part of the experiment is to test the magnitude and rate of this integration from a small number of transplants. As long as the transplants successfully reproduce, we will be able to use conservation genomics methods to conduct tests of fitness. To be clear: no more than 8 fish would be transferred into a single recipient stream, and no more than 48 fish in total would be transferred during the project. As described earlier in the DN, an additional donor stream (South Fork of Quartz Creek) has been added to the project, while Staubach Creek will no longer be considered a donor population owing to disease concerns.

Cited Literature

- Bouzat, J., J. Johnson, and J. Toepfer. 2009. Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. *Conservation Genetics* **10**:191-201.
- Frankham, R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology* **24**:2610-2618.
- Frankham, R., J. D. Ballou, M. D. B. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the probability of outbreeding depression. *Conservation Biology* **25**:465-475.
- Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler, and S. G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. *Aquaculture* **173**:117-129.
- Hedrick, P. W., and R. Fredrickson. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conservation Genetics* **11**:615-626.
- Hendry, A. P., and S. C. Stearns. 2004. *Evolution Illuminated: Salmon and their Relatives*. Oxford University Press, New York.
- Hogg, J. T., S. H. Forbes, B. M. Steele, and G. Luikart. 2006. Genetic rescue of an insular population of large mammals. *Proceedings. Biological sciences / The Royal Society* **273**:1491-1499.
- Houde, A. L. S., D. J. Fraser, P. O'Reilly, and J. A. Hutchings. 2011. Relative risks of inbreeding and outbreeding depression in the wild in endangered salmon. *Evolutionary Applications* **4**:634-647.
- Hwang, A. S., S. L. Northrup, D. L. Peterson, Y. Kim, and S. Edmands. 2012. Long-term experimental hybrid swarms between nearly incompatible *Tigriopus californicus* populations: persistent fitness problems and assimilation by the superior population. *Conservation Genetics* **13**:567-579.

- Johnson, W., D. Onorato, M. Roelke, E. D. Land, M. Cunningham, R. C. Belden, R. McBride, D. Jansen, M. Lotz, D. Shindle, J. Howard, D. E. Wildt, L. M. Penfold, J. A. Hostetler, M. K. Oli, and S. J. O'Brien. 2010. Genetic restoration of the Florida panther. *Science* **329**:1641-1645.
- Robinson, Z., J. A. Coombs, M. Hudy, K. H. Nislow, B. H. Letcher, and A. R. Whiteley. *in press*. Experimental test of genetic rescue in isolated populations of brook trout. *Molecular Ecology*.
- Tymchuk, W. E., L. F. Sundström, and R. H. Devlin. 2007. Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). *Evolution; international journal of organic evolution* **61**:1225-1237.
- Vila, C., A. K. Sundqvist, O. Flagstad, J. Seddon, S. Björnerfeldt, I. Kojola, A. Casulli, H. Sand, P. Wabakken, and H. Ellegren. 2003. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society B-Biological Sciences* **270**:91-97.
- Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. Genetic rescue to the rescue. *Trends in Ecology & Evolution* **30**:42-49.
- Wood, C. C. 1995. Life history variation and population structure in sockeye salmon. Pages 195-216 in J. L. Nielsen, editor. *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation*. American Fisheries Society Symposium 17:195-216, Bethesda, MD.